UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MEMORANDUM

SUBJECT: Review of Product Chemistry, Manufacturing Process, Toxicity studies and Waiver requests for Acute Toxicity Studies for section 3 registration of a TGAI Trianum Technical (EPA Reg. No. 89635-R) and two EPs Trianum G (EPA Reg. No. 89635-E) and Trianum WG (EPA Reg. No. 89635-G)

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THROUGH: John L. Kough, Ph.D., Senior Scientist
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TO: Shanaz Bacchus, Regulatory Action Leader
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ACTION REQUESTED: To review product chemistry, manufacturing process, formation of unintentional ingredients, analysis of samples, acute dermal irritation, acute eye irritation studies, and waiver requests for acute toxicity studies for the registration of Trianum Technical (EPA Reg. No. 89635-R) and two EPs Trianum G (EPA Reg. No. 89635-E) and Trianum WG (EPA Reg. No. 89635-G).

CONCLUSION: **UPGRADABLE** after the registrant has corrected the following:

1. Labels for the EPs should state that the product is stable for 6 months and should be kept refrigerated after reconstitution.

2. The label of Trianum G should state that the user should wear a dust mask when using this product.

CONTAINS FIFRA CONFIDENTIAL BUSINESS INFORMATION

DATA REVIEW RECORD

Active Ingredient:	*Trichoderma harzianum* Rifai, strain T-22
Product Names:	Trianum Technical, Trianum G, Trianum WG
Company Name:	Koppert Biological Systems, Inc. Howell, MI 48843.
Decision Numbers:	471288, 471289, 471290
Chemical Number:	119202
Submission Numbers:	925617, 925622, 925623
DP Barcodes:	409538, 409554, 409555
MRID Numbers:	48972401-06; 48971901- 04; 48972101 - 04: Product Identity, Manufacturing Process, Unintentional Ingredients; Analysis of Samples. 48972408, 09; 489906, 07; 48972106, 07: Acute Dermal & Eye Irritation. 48972410, 48971908, 48972108: Data waivers for Acute Toxicity Studies. 48972407, 48971905, 48972105: Hypersensitivity Incidents Reports.

BACKGROUND:

T-22 Technical is a manufacturing use product to be incorporated into end use products (EPs) as a biological fungicide to provide protection from plant root pathogens. *T. harzianum* is a general saprophytic fungus growing on vegetable matter. *Trichoderma* spp. are obligatory aerobic and prefer acidic pH levels. *T. harzianum* typically do not grow below 6°C and above 36°C. *T. harzianum* has been reported to promote plants and induce increase resistance to diseases and also to abiotic stresses such as nutrient and water deficiency. Different modes of action like competition with plant pathogens for space and substrates in the rhizosphere, mycoparasitism and the secretion of fungal cell wall degrading enzymes, production of antifungal substances, growth promotion, solubilization of inorganic nutrients, and induction of systemic resistance in plants are reported for *T. harzianum*.

DISCUSSION:

Trianum Technical (TGAI) is a manufacturing use product to be incorporated into end use products as a biological fungicide to provide protection from plant root pathogens. *Trichoderma harzianum* strain T-22 produced by Koppert BV was confirmed to be identical to the strain previously registered by Bioworks. [redacted] The nominal concentration and certified limits for the T-22 Technical and the EPs are given on the CSF. The levels of microbial impurities in the formulated product are acceptable.

RECOMMENDATION: **UPGRADABLE** after the registrant has corrected the following:

1. Labels for the EPs should state that the product is stable for 6 months and should be kept refrigerated after reconstitution.
2. The label of Trianum G should state that the user should wear a dust mask when using this product.

Manufacturing process information may be entitled to confidential treatment

SUMMARY OF DATA SUBMITTED:

Product Chemistry, Manufacturing Process, Formation of Unintentional Ingredients, Analysis of Samples, & Certification of Limits (MRID Nos. 48972401 – 06; 48971901- 04; 48972101 - 04)

Product Identity: *T. harzianum* is a general saprophytic fungus growing on vegetable matter. *T. harzianum* occurs world-wide in cold, but mainly moderate to tropical soils, preferring warm climates. *Trichoderma* spp. are obligatory aerobic and prefer acidic pH levels. *T. harzianum* typically do not grow below 6°C and above 36°C. T-22 does not grow at all at 37°C tested on agar plates for 7 days. *T. harzianum* has been reported to promote plants and induce increase resistance to diseases and also to abiotic stresses such as nutrient and water deficiency. *Trichoderma harzianum* strain T-22 produced by Koppert BV was confirmed to be identical to the strain previously registered by Bioworks.
Deficiencies: None.

Manufacturing Process:

[redacted]

Deficiencies: None

Discussion of Formation of Unintentional Ingredients: The maximum accepted levels of microbial impurities in the formulated product are: [redacted]

[redacted] Results of these tests for several batches produced in 2009-2012 are shown in Table 1.

Table1. Analyses of contaminants in Trianum Technical, Trianum WG and Trianum G.

[redacted]

Deficiencies: None.

Analysis of Samples:

Trianum Technical

No.	Component	Function	g/kg	g/kg	%-w/w
[redacted]					

[redacted]

Trianum WG

No.	Component	Function	g/kg	g/kg	%
[redacted]					

Trianum G

No.	Component	Function	g/kg	g/kg	%
[redacted]					

Deficiencies: None.

Certification of Limits: Table 2 lists the nominal concentration and certified limits for the T-22 Technical and the End Use Products (EPs) as given on the CSF. The certified limits are within the OPPTS 830.1750 recommended ranges.

Table 2. Certified limits of Trianum Technical, Trianum G, and Trianum WG

Ingredients		Concentration (% by weight)		
		Nominal	Lower	Upper
Trianum Technical (> 1 x 10^9 cfu/g)	TGAI	13.65	12.90	14.30
Trianum G	EP	1.32	1.25	1.39
Trianum WG	EP	3.65	3.47	3.83

Deficiencies: None.

Physical and Chemical Properties of Trianum Technical

TABLE 3. Physical and Chemical Properties for Trianum Technical			
Guideline Reference No./Property		Description of Result	Methods
830.6302	Color	Grey or green	Visual inspection
830.6303	Physical State	Powder	Visual inspection
830.6304	Odor	Earthy	Olfactory inspection
830.6313	Stability	Unstable at elevated temperatures; stable to metals or metal ions (Fe, Fe^{+3}, Al, Al^{+3})	Elevated temperature to 54°C resulted in 98.7% loss of viability of T-22 based on CFU counting[b]
830.6314	Oxidation/Reduction: Chemical Incompatibility	Not applicable, the product does not contain oxidizing or reducing agents.	
830.6315	Flammability	Not applicable, the product does not contain flammable ingredients.	
830.6316	Explodability	Not applicable, the product does not contain explosive ingredients.	
830.6317	Storage Stability	Stable at 21°C for 12 months	CFU counting during a 12-month storage stability study in LDPE packaging material
830.6319	Miscibility	Not applicable, the product is not an emulsifiable liquid to be mixed with petroleum solvents.	
830.6320	Corrosion Characteristics	Not corrosive to LDPE packaging material.	12-month storage stability study in LDPE packaging material @ 21°C
830.6321	Dielectric Breakdown Voltage	Not required for TGAI/MP	
830.7000	pH	4.88	Measured in 1% aqueous suspension at room temperature (25°C) with a pH meter.
830.7100	Viscosity	Not applicable, the product is a solid	
830.7200	Melting Range	Not applicable, the product is not a pure active ingredient.	
830.7220	Boiling Range	Not applicable, the product is a solid.	
830.7300	Density/Relative Density/Bulk Density	0.159 g/cm^3	CIPAC MT-33
830.7370	Dissociation Constant in Water	Not applicable, the product is not a pure active ingredient.	
830.7550	Partition Coefficient	Not applicable, the product is not a pure active ingredient.	
830.7840	Water Solubility	Not applicable, the product is not a pure active ingredient.	
830.7950	Vapor Pressure	Not applicable, the product is not a pure active ingredient.	

Deficiencies: Labels for the EPs should state that the product is stable for 6 months and should be kept refrigerated after reconstitution.

Acute Dermal and Eye Irritation Toxicity Studies

1- Trianum Technical

In a primary dermal irritation study (MRID #48972409), 2 male/1 female NZW albino rabbits, 12 weeks-6 months of age and 2-4 kg at dosing, were dermally exposed to 0.5 mL of Trianum Technical, undiluted, to ~8 x 8 in. of body surface area. Test sites were covered with a semi-occlusive dressing for 4 hours. Animals were then observed for 72 hours. Irritation was scored by the method of Draize. In this study, Trianum Technical is not a dermal irritant based on 0.0 PII Score. This study is classified as **Acceptable.**

In a primary eye irritation study (MRID # 48972408), 0.1 mL by volume of Trianum Technical was instilled into the conjunctival sac of the right eye of 2 male/1 female NZW albino rabbits, ~12 - 14 weeks of age and 2.5-2.7 kg at dosing, for 1 second. Eyes were washed for 1 minute at 24 hours after dosing. Animals were observed for 72 hours. Irritation was scored by the method of Draize. In this study, Trianum Technical is not an irritant to the eye based on Max Score 0.0. , and is considered as EPA Toxicity Category IV. This study is classified as **Acceptable.**

2- Trianum G

In a primary dermal irritation study (MRID # 48971907), 1 males and 2 female New Zealand White Albino rabbits were dermally exposed to 500 mg of test substance moistened with 0.5 mL of deionized water on the dorsal area of the trunk clipped to expose an area of at least 8 x 8 cm. The test substance was maintained in contact with the skin for 4 hours. Animals were then observed for 3 days. Irritation was scored by the method of the Draize. Based on the scores at the 72-hour observation only, the test substance is assigned to Toxicity Category IV. This study is classified as **Acceptable.**

In a primary eye irritation study [MRID # 48971906], 100 mg of Trianum G (Granule) was instilled into the conjunctival sac of right eyes of 2 males and 1 female New Zealand White Albino rabbits for 24 hours. All treated eyes were washed with room temperature deionized water for one minute immediately after recording the 24-hour observation. Animals were then observed for 3 days. Irritation was scored by the method of Draize. There were no positive effects exhibited in any eyes at 72 hours after treatment. The test substance is rated minimally irritating and assigned to Toxicity Category III. This study is classified as **Acceptable.**

3- Trianum WG

In a primary dermal irritation study (MRID 48972107), 2 male and 1 female New Zealand White Albino rabbits were dermally exposed to 500 mg of test substance moistened with 0.5 mL of deionized water on the dorsal area of the trunk clipped to expose an area of at least 8 x 8 cm. The test substance was maintained in contact with the skin for 4 hours. Animals were then observed for 3 days. Irritation was scored by the method of the Draize. Based on the primary irritation index of 0.0, the test substance is rated non-irritating. Based on the scores at the 72-hour observation only, the test substance is assigned to Toxicity Category IV. . This study is classified as **Acceptable.**

In a primary eye irritation study (MRID # 48972106), 100 mg of Trianum WG was instilled into the conjunctival sac of right eyes of 1 males and 2 female New Zealand White Albino rabbits for 24 hours. Animals were then observed for 3 days. Irritation was scored by the method of Draize. There were no positive effects exhibited in any eyes after treatment. The test substance is rated non-irritating and assigned to Toxicity Category IV. This study is classified as **Acceptable.**

Waiver Requests for Acute Toxicity Studies & Hypersensitivity Incidents Reports
(MRID Nos. 48972410, 48971908, 48972108, 48972407, 48971905, 48972105)

1-Acute Oral Toxicity (OPPTS 870.1100)
The waiver request is based on the following rationale:

1. This study is required only if inert ingredients may pose any significant health effects in combination with the active ingredient.
2. Trianum Technical and EPs contains two inert ingredients; both are common food ingredients. Skin and eye irritation studies completed with Trianum Technical and EPs indicate no toxicity. Responses in both studies are consistent with EPA Toxicity Category IV.
3. An acute oral toxicity/pathogenicity study has been completed with *Trichoderma harzianum* Rifai Strain T-22 (MRID #41245904) which is identical to the current TGAI.

2- Acute Dermal Toxicity (OPPTS 870.1200)
The waiver request is based on the following rationale:

1. Trianum TGAI & EPs contain two inert ingredients; both are common food ingredients.
2. Skin and eye irritation studies completed with Trianum Technical, Trianum G, and Trianum WG indicate no toxicity. Responses in both studies are consistent with EPA Toxicity Category IV.
3. The Trichoderma harzianum Rifai Strain T-22 in Trianum Technical is identical to the Trichoderma harzianum Rifai Strain T-22 (ATCC 20847) registered by Bioworks Inc.

3- Acute Inhalation Toxicity (OPPTS 870.1300)
The waiver request is based on the following rationale:
1. This study is required only if it contains inert ingredients which may pose any significant health effects in combination with the active ingredient. Trianum Technical contains two inert ingredients which are common food ingredients.
2. When the product consists of, or under conditions of use would result in an inhalable material. These conditions do not apply to Trianum Technical.
3. Skin and eye irritation studies completed with Trianum TGAI & EPs indicate no toxicity from the technical; responses in both studies are consistent with EPA Toxicity Category IV.

4- Hypersensitivity Incidents (OPPTS 885.3400)
This guideline requirement states that all incidences of hypersensitivity must be reported to US EPA. No incidences of employee hypersensitivity to the Koppert's Trianum products have been documented in production and office facilities. Approximately 18 employees are directly involved in Koppert's production of strain T-22. In the future, if any occur, these incidences will be reported as specified in the Pesticide Incident Report form (EPA Form Number 8550-5, OMB Form number 158-R0008).

CLASSIFICATION: **ACCEPTABLE,** however, the following restriction is applicable to Trianum G: The label of Trianum G should state that the user should wear a dust mask when using this product. Alternatively, the registrant should submit an acute inhalation toxicity study because of the [redacted] in the product.